

SCIENCE CHINA
Life Sciences

• RESEARCH HIGHLIGHT •

June 2014 Vol.57 No.6: 645–646

doi: 10.1007/s11427-014-4659-5

Structure of the human P2Y₁₂ receptor in complex with an antithrombotic drug

DU Bing & LIU MingYao*

Shanghai Key Laboratory of Regulatory Biology, Institute of Biomedical Sciences and School of Life Sciences, East China Normal University, Shanghai 200241, China

Received April 9, 2014; accepted April 15, 2014; published online May 5, 2014

Citation: Du B, Liu MY. Structure of the human P2Y₁₂ receptor in complex with an antithrombotic drug. *Sci China Life Sci*, 2014, 57: 645–646, doi: 10.1007/s11427-014-4659-5

After the 2012 Nobel Prize in Chemistry was awarded for the study of G-protein-coupled receptors (GPCRs), renewed interest was evident within the field. As seven-transmembrane (7TM) receptors with crucial physiological and pathological functions, GPCRs are the most popular drug targets known. In recent years, the Chinese government has funded several major projects on basic GPCR research and new drug discovery research targeting GPCRs through the National Basic Research Program (973 Program) and National Scientific and Technological Major Project for “Significant New Drugs Creation and Manufacturing Program”. With the support from 973 Program (2012CB910400), Dr. Zhao Qiang’s group from the Shanghai Institute of Materia Medica reported the high resolution (2.7 Å) crystal structure of P2Y₁₂R in complex with a non-nucleotide reversible antagonist, AZD1283, in *Nature* [1]. Their findings provide the ground work for P2Y₁₂R- based drug research and development.

Purinergic P2Y receptors which belong to the group of metabotropic P2Y purinergic GPCRs stimulated by nucleotides, play crucial roles in inflammation, cancer and cardiovascular diseases. There are two distinct P2Y receptors for ADP expressed on platelets: the Gq-coupled P2Y₁R and the Gi-coupled P2Y₁₂R. Both contribute to collagen-induced platelet microparticle formation in whole blood and the formation of platelet-leukocyte aggregates. However, only P2Y₁₂R is involved in the exposure of phosphatidylserine

by thrombin or other platelet agonists [2]. P2Y₁₂R (formerly SP1999) was discovered and identified as an ADP receptor with platelet clopidogrel sensitivity in 2001 [3]. Several generations of P2Y₁₂R-targeted antithrombotic drugs have been developed because of its critical functional role in platelet aggregation. The first generation drugs, such as ticlopidine, clopidogrel and prasugrel, were prodrugs which needed to be metabolized before covalently binding to P2Y₁₂R. Now newer drugs including ticagrelor, cangrelor and elinogrel that are either approved by the FDA or are still in clinical trials, directly act on P2Y₁₂R. However, clinical studies have revealed certain limitations of these drugs including their long half-life and/or significant side effects. There is an unfulfilled medical need to develop new generation P2Y₁₂R inhibitors, which was previously severely limited because of a lack of receptor structural and biochemical information.

Dr. Zhao’s group modified the protein sequence of the P2Y₁₂R by inserting the thermostabilized apocytochrome b₅₆₂RIL (BRIL) into the third intracellular loop region of the receptor, and incorporating a D294^{7.49}N (superscript Baltes-Winstein nomenclature) mutation in the N[D]P^{7.50}xxY motif of the seventh transmembrane region to improve the protein yield and facilitate crystallization. The crystal structure of the antagonist AZD1283 (a novel P2Y₁₂R antagonist developed for inhibition of artery thrombosis by AstraZeneca) bound to P2Y₁₂R was solved for a crystal with C2 space group.

The P2Y₁₂R structure contains the typical seven trans-

*Corresponding author (email: myliu@bio.ecnu.edu.cn)

membrane helices (as shown in Figure 1). Helix VIII in the C-terminal part is well resolved and is parallel to the lipid bilayer. The only observed disulfide bond is formed between C17 in the N-terminal part and C270^{7,25}, while the conserved disulfide bond between C97^{3,25} and C175^{ECL2} is flexible and thus, was not modeled [4]. Two cholesterol molecules bound to each receptor are observed, suggesting the potential role of cholesterol in P2Y₁₂R mediated signal transduction. When compared with those of other known GPCRs, the structures of the V and VI transmembrane helices are significantly different in P2Y₁₂R [5].

The AZD1283 binding pocket is composed of residues from helix III to helix VII. This pocket was previously only found in the GPCR PAR1. As both receptors (P2Y₁₂R and PAR1) belong to the δ subgroup of GPCRs, this binding pocket may contain features that are common within the δ subgroup of GPCRs. A series of amino acid side chains in the helices are involved in polar and hydrophobic interac-

tions with the ligand. The benzene ring of Y105^{3,33} interacts with the nicotinate of AZD1283 via a π - π interaction, while it has a hydrophobic interaction with the piperidine group. The phenyl group of AZD1283 is accommodated by the hydrophobic pocket formed by F252^{6,51}, R256^{6,55}, Y259^{6,58}, L276^{7,31} and K280^{7,35}.

Besides the AZD1283 binding site, there is another binding pocket observed in the crystal structure of P2Y₁₂R, which is composed of helices I-III and VII. This is the first time that two different extracellular binding sites have been observed in a GPCR structure. Docking assays suggest that the AZD1283 binding pocket interacts with reversible ligands, while the other pocket may favor covalent small molecules. This indicates that an allosteric modification mechanism may play a role in regulating P2Y₁₂R signaling. According to the docking analysis, C97^{3,25}, which is thought to form a conserved disulfide bond with C175^{ECL2}, is probably the covalent binding site of an active metabolite. This correlates well with the labile disulfide bond observed in the structure of P2Y₁₂R; the active metabolites are reactive thiols, which would react with free cysteines rather than disulfides in the receptor. As P2Y₁₂R is an important drug development target, its structure provides valuable insights for the development of improved P2Y₁₂R antagonists. The unique conformation of the P2Y₁₂R structure deepens our understanding of the ligand recognition elements of the purinergic receptor and the biological functions of GPCRs. Future structural studies of P2Y₁₂R that build on the information described herein will support the design of new drugs with reduced side effects.

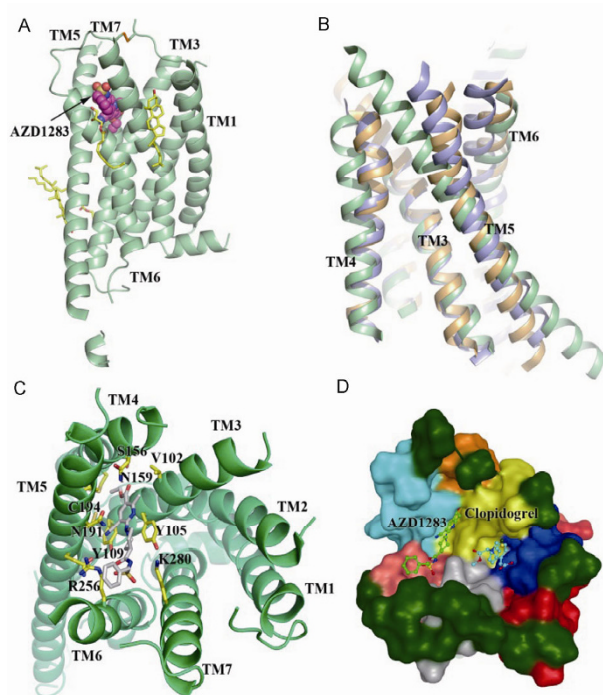


Figure 1 Overview of the P2Y₁₂R-AZD1283 complex structure and ligand-binding for AZD1283. A, Cartoon representation of P2Y₁₂R. P2Y₁₂R is shown as a green ribbon. AZD1283 is shown as magenta spheres. Cholesterol and lipids are yellow carbon stick structures. B, Views of P2Y₁₂R (green) compared with β_2 AR (PDB accession 2RH1, brown) and PAR1 (PDB accession 3VW7, blue). C, Key residues in P2Y₁₂R for AZD1283 binding. AZD1283 (gray carbons) and receptor residues (yellow carbons) involved in ligand binding are shown as stick structures. D, The two pocket binding model of P2Y₁₂R. The ligand AZD1283 and docking pose of the active metabolite of clopidogrel are shown as green carbon stick structures.

- 1 Zhang K, Zhang J, Gao ZG, Zhang D, Zhu L, Han GW, Moss SM, Paoletta S, Kiselev E, Lu W, Fenalti G, Zhang W, Müller CE, Yang H, Jiang H, Cherezov V, Katritch V, Jacobson KA, Stevens RC, Wu B, Zhao Q. Structure of the human P2Y₁₂ receptor in complex with an antithrombotic drug. *Nature*, 2014, doi: 10.1038/nature13083
- 2 Cattaneo M. Bleeding manifestations of congenital and drug-induced defects of the platelet P2Y₁₂ receptor for adenosine diphosphate. *Thromb Haemost*, 2011, 105(Suppl 1): S67-74
- 3 Hollopeter G, Jantzen HM, Vincent D, Li G, England L, Ramakrishnan V, Yang RB, Nurden P, Nurden A, Julius D, Conley PB. Identification of the platelet ADP receptor targeted by antithrombotic drugs. *Nature*, 2001, 409: 202-207
- 4 Hanson MA, Roth CB, Jo E, Griffith MT, Scott FL, Reinhart G, Desale H, Clemons B, Cahalan SM, Schuerer SC, Sanna MG, Han GW, Kuhn P, Rosen H, Stevens RC. Crystal structure of a lipid G protein-coupled receptor. *Science*, 2012, 335: 851-855
- 5 Rasmussen SG, DeVree BT, Zou Y, Kruse AC, Chung KY, Kobilka TS, Thian FS, Chae PS, Pardon E, Calinski D, Mathiesen JM, Shah ST, Lyons JA, Caffrey M, Gellman SH, Steyaert J, Skiniotis G, Weiss WI, Sunahara RK, Kobilka BK. Crystal structure of the β_2 adrenergic receptor-Gs protein complex. *Nature*, 2011, 477: 549-555